A report on

Studies on performance of shoot tip grafting of various citrus species on rough lemon (*Citrus jambhiri* Lush.) rootstock

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CERTIFICATE

This is to certify that synopsis titled "**Studies on performance of shoot tip grafting of various citrus species on rough lemon (*Citrus jambhiri* Lush.) rootstock**" submitted in partial fulfillment of the requirement for the award of degree of Master of Science in Agriculture in the discipline of Horticulture (Fruit Science), is a research work carried out by Sanyogita (Registration No. 11717856) under my supervision and that no part of this synopsis has been submitted for any other degree or diploma.

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INTRODUCTION

Citrus is one of the most important fruit crops. Peoples consume as a fresh fruit and as a salad purpose. Majority of the citrus fruits are native to tropical and subtropical regions. In India citrus rank third in area and production after the mango and banana (Ghosh, 1997). India is the sixth largest citrus producer of the world (Anonymous, 1997).

Citrus is botanically known as citrus reticulate, genus citrus L. and belongs to family Rutaceae. Chromosome number of citrus is (2n=2x=18). Citrus belongs to class dicotyledoneae, sub class Archichlamydeae, order geraniales and sub order is garaniineae. According to hogson, different citrus species are valid like: the acid members (citron, lemon, lime); the orange group (myrtle- leaf orange, sour orange, sweet orange); the pummel-grapefruit group (grapefruit, chakotra); the mandarin group (sangtra, satsuma mandarin) papeda group (ichang papeda, khasi papeda alemow), other species (rangpur lime, calamondin, indian wild orange). Only 8 citrus species are generally cultivated for edible purposes are: *C. sinensis, C. reticulata, C. aurantium, C. limon, C. aurantifolia, C. grandis, C. paradisi*, and *C. medica*.

Citrus grown in India, 9.15 lakh and total production 79.22 lakh tonnes annually. In Punjab, annual production of citrus fruit is 10.16 lakh tonnes. Citrus fruit rich source of vitamin C. the rind of the citrus rich in pectin and essential oils. Most of the citrus fruits such as sweet orange grapefruit and mandarins are taken as fresh fruits and also use as a salad, juices, squashes, cocktails, syrup, concentrate marmalades and pickle (J.S. Bal).

Different types of citrus rootstocks are: sour orange, sweet orange, rough lemon, trifoliate orange, citranges, rangpur lime, Cleopatra mandarin.

Sour orange: It is hardy, vigorous and deep rooted in nature. It is resistant to drought, foot rot and viruses as well as susceptible to tristeza virus and scab. The skin of the fruit is thin, smooth, and produces high quality juice.

Sweet orange: It is shallow rooted rootstock and producing large vigorous productive tree. It is susceptible to foot rot and resistant to tristeza and exocortis.

Trifoliate orange: It is a dwarfing rootstock. Fruit is small in size but excellent quality of the fruits. It is susceptible to canker, scab and exocortis and resistant to foot rot, tristeza and nematodes.

Citranges: It is a hybrid of sweet orange and trifoliate orange. It is resistant to tristeza and foot rot and susceptible to exocortis. They are dwarf and highly polyembryonic.

Rangpur lime**:** It is a vigorous productive trees and hardy rootstock. It is resistant to tristeza and drought conditions but susceptible to exocortis.

Cleopatra Mandarin: The size of the fruit is small. It is tolerant to tristeza, exocortis and foot rot.

Rough Lemon: Rough lemon (*Citrus jambhiri* Lush.) is the common rootstock. In Punjab, rough lemon is known as 'Jatti khatti'. It is a shrub tree and height up to 10 ft. The branches of tree spreading and spinous habit and bark of the tree is green. Leaves are unifoliolated in nature, reddish or purplish in colour and petioles are wingless. At the apex of the leaves is articulate with base of blade; leaflet blades are oblong, base of obtuse rounded. Inflorescences of citrus are cyme. Flowers are pedicellate and staminate, 5-merous; bud of the flower is purplish and white inside in colour. Calyx of flower light green. Stamen are pubescent, anthers are oblong and length 3-4 mm, anther colour greenish yellow. Thin style up to 2.5 mm long and globose stigma. Fruits are spherical, small to medium-sized. Fruit of this rootstock is more acidic. It is tolerant to tristeza and susceptible to foot rot and scab. It shows highly polyembryony.

Grafting is asexual propagation method. The scion and rootstock are connected in proper way they may joint and develop successful plant. The upper part of the plant is scion and lower part of the plant is known as rootstock. The grafting can be done on 1-2 year old rootstock. Select the scion shoot of matching thickness rootstock. When the grafted plant start growing, the above graft union portion of rootstock is removed. In tropical and sub-tropical fruits, grafting gives better result in June- July.

In vitro shoot tip grafting (STG) is a miniature grafting which involves grafting a minute shoot tip (0.1 to 0.3 mm) on two-week-old seedling rootstock performed under aseptic conditions. It produces true to type, non-juvenile disease-free plants unlike nucellar embryony in vitro or in vivo. This technique is a prerequisite for cleaning the citrus cultivars /indigenous collections from diseases (virus, virus like diseases and greening bacteria), since absolutely there is no control measure once the virus enters the plant system except shoot tip grafting (STG).

Micro budding is a miniature budding on young citrus root stocks measuring 3 mm diameter in which the bud is inserted on the detopped root stock in a wedge cut and immediately

protected by covering with a micropipette tip. After a week, micro buds are observed and then micro tips are removed after their sprouting within 12-14 days. Micro budding facilitates faster propagation with reduced cost. This biotech break has a tremendous scope in commercial propagation and research. It reduces huge cost on labour and maintenance during the commercial propagation on low cost green house. It can be utilized in biological indexing of virus, viroids, greening bacterium and other disease inoculation and expression studies at much faster pace, enable year-round multiplication and shortens the nursery phase.

Considering the significance of shoot tip grafting under in-vitro condition and microbudding the proposal has been developed to perform shoot tip grafting practice on 6 months old stock of jambhiri with the following objectives:

1. To evaluate the success of shoot tip grafting in vivo condition.
2. To determine the compatibility of various scion cultivars for Jambhiri rootstock.

REVIEW OF LITERATURE

Micrografting is a relatively new grafting technique and consists of grafting an apex taken from a mother plant on to (a) a young greenhouse or nursery grown plant in accordance with accepted grafting techniques (in vivo micrografting); (b) a decapitated young plant grown from a seedling under aseptic conditions, or a microcutting obtained from in vitro vegetative multiplication (in vitro micro-grafting). Following early experiments by Doorenbos in 1953 in ivy, and later by Holmes (1956) on chrysanthemum, these two variations of the micrografting techniques have been used in particular on woody species, and especially on fruit trees, where work was carried out on different species of the lemon tree with a view to eliminating various viral diseases (**Murashige *et al.***, 1972, **Roistacher *et al.***, 1976, **Roistacher and Kitto** (1977) and on *Hevea* (**Muzik and Cruzada**, 1958).

Chand *et al.* (2013) studied that the diseases of the citrus can be eliminated by in vitro shoot tip grafting. In citrus, some diseases like citrus exocortis and stybbom are not easily eliminate from the mother plants, which can be eliminated by in vitro shoot tip grafting.

Kamanga *et al*. (2013) they studied at the Lilongwe University of Agriculture and Natural Resources (LUANAR) in Malawi to examined the comparative success of budding and grafting. Grafted plants take more buds as compare to budding plants. shoot girth, number of leaves and number of branches are also affected by no. of buds. Between number of branches and number of leaves shows linear relationship and positive correlationship was studied.

Alam *et al*. (2006) observe in Sweet lime, Grapefruit cv. Sham bar and Mandarin cv. Feutrell's microbudding technique was survey for early age propagation under protected environments. Best result shows in sweet lime (76 %) followed by grapefruit (68 %) and Feutrell's early (44.0%). No significant differences were recorded in plant height, stem girth of grapefruit is higher as comare to other citrus species. But in sweet lime, leaf blade length (8.76 cm) is maximum and minimum (5.76 cm) in Feutrell's early.

Singh *et al*. (2013) observe shoot tip grafting in Nagpur Mandarin. The maximum graft success 53.33%, 40.00% and 43.33%, respectively were observed over the rootstocks; namely, They observe maximum success in Carrizo (at 13 days old) , in Rangpur lime (at 14 days old) and in Rough lemon (at 16 days old).

PROPOSED RESEARCH OBJECTIVES

Considering the significance of shoot tip grafting under in-vitro condition and microbudding the proposal has been developed to perform shoot tip grafting practice on 6 months old stock of jambhiri with the following objectives:

1. To evaluate the success of shoot tip grafting under in vivo condition.
2. To determine the compatibility of various scion cultivars for Jambhiri rootstock.

MATERIALS AND METHODOLOGIES

The study will be carried out at Departmental Nursery, Agricultural Farm, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The details of materials used and methodology adopted has be described below:

1. Raising of rootstocks of Jambhiri
2. Shoot tip grafting in month of July.
3. Scion cultivars will be collected from Centre of Excellence in Citrus, Hosiarpur. The 20 scions of each of the following 15 cultivars (Exotic and Indigenous) will be taken for study:

   Michal, Murcott, Daisy, Fairchild, Freemont, W-murcott, Pearl-Tangelo, Santra, Lemon & Kinnow.
4. Treatments: 10 (Scion cultivars)
5. Replication: 3
6. Number of plants in each replication: 10
7. Total stocks: 10 x 3 x 10 = 300

8. Observations to be recorded

S. No.	Observations
1	Date of grafting
2	Height of grafting
3	No. of days taken for successful grafting
4	Growth of rootstock at interval of 10 days till April
5	Growth of scion at interval of 10 days till April
6	Diameter of stock at interval of 10 days till April
7	Diameter of scion at interval of 10 days till April
8	Scion: Stock ratio (Length wise and diameter wise)
9	Internodal length of scion
10	No. of leaves
11	No. of branches
12	Percent success of grafting
13	Percent survival
14	Chlorophyll content of leaves
15	Leaf area

Morphological characterization involves recording variation in habit, leaf, flower, and fruit traits (Dale 1996). Morphological characters traditionally identified crop species and varieties (Nielsen and Lovell 2000). In the United States and Europe, morphological markers are used in addition to isozyme markers in plant patent descriptions (Nielsen and Lovell 2000). The current study also includes evaluation graft compatibility through certain molecular parameters and will cover following steps:

i. DNA extraction

ii. Marker development

iii. CAPS procedure

iv. Polymorphisms among cultivars and detection as markers

v. Cultivar identification and reproducibility

The study will be conducted by using following markers:

TAA15	F: GAAAGGGTTACTTGACCAGGC
	R: CTTCCCAGCTGCACAAGC
TAA27	F: GGATGAAAAATGCTCAAAATG
	R: TAGTACCCACAGGGAAGAGAGC
TAA41	F: AATGCTGAAGATAATCCGCG
	R: TGCCTTGCTCTCCACTCC
CAC23	F: ATCACAATTACTAGCAGCGCC
	R: TTGCCATTGTAGCATGTTGG
CAC15	F: TAAATCTCCACTCTGCAAAAGC

	R: GATAGGAAGCGTCGTAGACCC
CAC33	F: GGTGATGCTGCTACTGATGC
	R: CAATTGTGAATTTGTGATTCCG
CAC39	F: AGAAGCCATCTCTTCTGCTGC
	R: AATTCAGTCCCATTCCATTCC

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